

FIG. 1

HSP 90-beta (Mouse)  
SEQ ID NO:1

```

1 mpeevhhgee evetfafgae iaqlmsliin tfysnkeifl relisnasda ldkiryeslt
61 dpskldsgke lkidiipnpq ertltlvdtg igmtkadlin nlgtiaksgt kafmealqag
121 adismigqfg vgfysaylva ekvvvitkhn ddegyawess aggsftvrad hgepigrgtk
181 vilhlkedqt eyleerrvke vvkhsqfig ypitlyleke rekeisddea eeekgekeee
241 dkddeekpki edvgsdeedd sgdkkkkktk kikekyidqe elnktkpiwt rnpdditqee
301 ygefysltn dwedhlavkh fsvegglefr allfiprrap fdlfenkkkk nniklyvrrv
361 fimdsodeli peylnfirgv vdsedlplni sremllqgski lkvirknivk kclelfsela
421 edkenykkfy eafsknlklg ihedstnrrr lsellryhts qsgdemtsls eyvsrmketg
481 ksiyyitges keqvansafv ervrkrgefv vymtepiday cvqqlkefdg kslvsvtkeg
541 lelpedeeek kkmeeskakf enlcklmkei ldkkvekvti snrlvsspc ivtstygwta
601 nmerimkaqa lrdnstmgym makkhleinp dhpivetlrq kaeadkndka vkdlvllfe
661 tallssgfs l edpqthsnri yrmiklglgi dedevaaep naavpdeipp legdedasrm
721 eevd

```

HSP 90-beta (Human)  
SEQ ID NO:2

```

1 mpeevhhgee evetfafgae iaqlmsliin tfysnkeifl relisnasda ldkiryeslt
61 dpskldsgke lkidiipnpq ertltlvdtg igmtkadlin nlgtiaksgt kafmealqag
121 adismigqfg vgfysaylva ekvvvitkhn ddegyawess aggsftvrad hgepigrgtk
181 vilhlkedqt eyleerrvke vvkhsqfig ypitlyleke rekeisddea eeekgekeee
241 dkddeekpki edvgsdeedd sgdkkkkktk kikekyidqe elnktkpiwt rnpdditqee
301 ygefysltn dwedhlavkh fsvegglefr allfiprrap fdlfenkkkk nniklyvrrv
361 fimdsodeli peylnfirgv vdsedlplni sremllqgski lkvirknivk kclelfsela
421 edkenykkfy eafsknlklg ihedstnrrr lsellryhts qsgdemtsls eyvsrmketg
481 ksiyyitges keqvansafv ervrkrgefv vymtepiday cvqqlkefdg kslvsvtkeg
541 lelpedeeek kkmeeskakf enlcklmkei ldkkvekvti snrlvsspc ivtstygwta
601 nmerimkaqa lrdnstmgym makkhleinp dhpivetlrq kaeadkndka vkdlvllfe
661 tallssgfs l edpqthsnri yrmiklglgi dedevaaep naavpdeipp legdedasrm
721 eevd

```

HSP 90-alpha (Human)  
SEQ ID NO:3

```

1 mpeetqtqdg pmeeevetf afgaeiaqlm sliintfysn keiflrelis nssdaldkir
61 yesltdpskl dsgkelhinl ipnkqdrtilt ivdtgigmtk adlinnlgti aksgtkafme
121 alqagadism igqfgvgfys aylvaekvtv itkhnnddeg awessaggsf tvrttdtgepm
181 grgktvilhl kedqteylee rrikeivkhh sqfigypitl fvekerdkev sddeaeeked
241 keeekkeek esedkpeied vgsdeeeekk dgdkkkkkki kekyidqeel nktkpiwtrn
301 pdditneeyg efysltn dwedhlavkhfs vegglefral lfvprrrapfd lfenrkkknn
361 iklyvrrvfi mdnceelipe ylnfirgvvd sedlplnistr emlqgskilk virknlvkcc
421 lelftelaed kenykkfyeg fskniklgih edsqrkkls ellryytsas gdemvslkdy
481 ctrmkenqkh iyyitgetkd qvansafver lrkhgleviy miepidaycv qqlkefegkt
541 lvsvtkegle lpedeeekkk geekktkfen lckimkdile kkvekvvsn rlvtsppciiv
601 tstygtanm erimkaqalr dntstmgymaa kkhleinpdh siietlrqka eadkndksvk
661 dlillyeta llssgfsled pqthanriy miklglgide ddptaddtsa avteempple
721 gdddtarmee vd

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2/25

HSP 84 (Mouse)  
SEQ ID NO:4

```
1 mpeevvhgee evetfafgae iaqlmsliin tfysnkeifl relisnasda ldkiyeslt
61 dpskldsgke lkidiipnpq ertltlvdtg igmtkadlin nlgtiaksgt kafmealqag
121 adismigqfg vgfysaylva ekvvvitkhn ddegyawess aggsftvrad hgepigrgtk
181 vilhlkedqt eyleerrvke vvkhsqfig ypitlyleke rekeisddea eeekgekeee
241 dkedeeekpi edvgsdeedd sgdkdkkktk kikekyidqe elnktkpiwt rnpdditqee
301 ygefysltn dwedhlavkh fsveggflef allfiprrap fdlfenkkkk nniklyvrrv
361 fimdscdeli peylnfirgv vdsedlplni sremllqgski lkvrknivk kclelfsela
421 edkenykkfy eafsknlklg ihedstnrrr lsellryhts qsgdemtsls eyvsmketq
481 ksiyyitges keqvansafv ervrkrgefv vymtepiday cvqqlkefdg kslvsvtkeg
541 lelpedeeek kkmeeskakf enlcklmkei ldkkvekvti snrlvsspc ivtstygwta
601 nmerimkaqa lrdnstmgym makkhleinp dhpivetlrq kaeadkndka vkdlvllfe
661 tallssgfsl edpqthsnri yrmiklglgi dedevtaeep saavpdeipp legdedasrm
721 eevd
```

HSP 84 (Human)  
SEQ ID NO:5

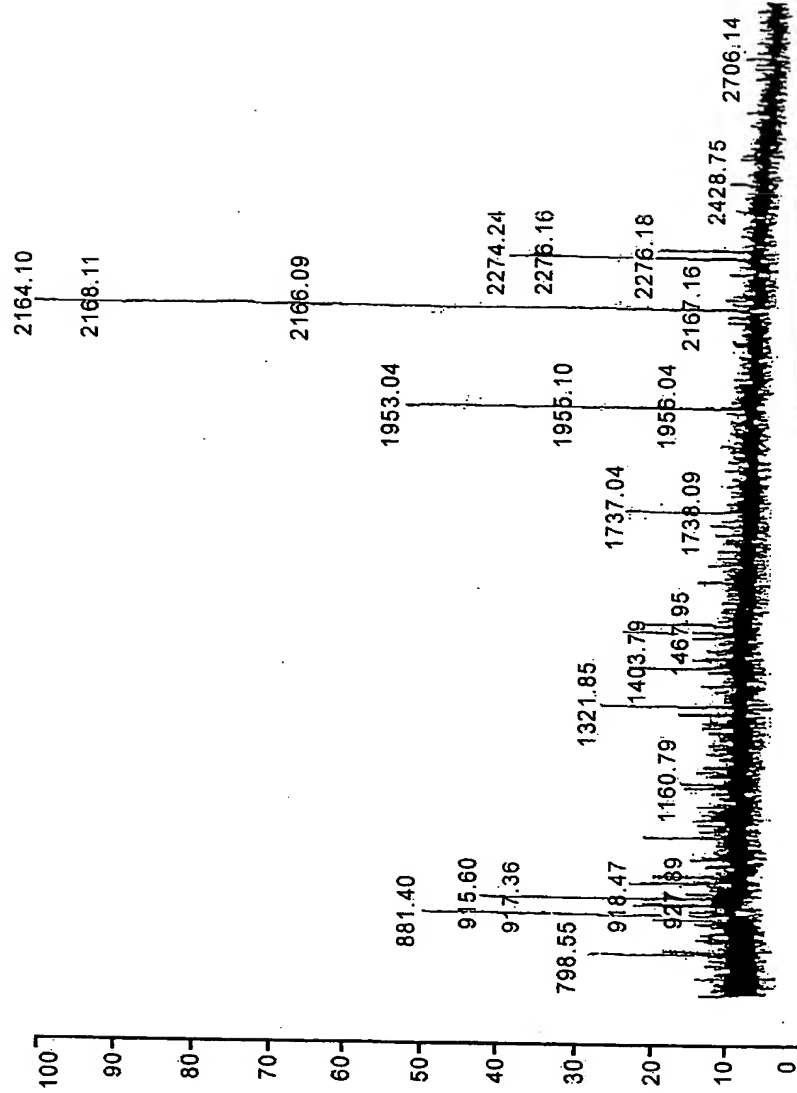
```
1 mpeevvhgee evetfafgae iaqlmsliin tfysnkeifl relisnasda ldkiyeslt
61 dpskldsgke lkidiipnpq ertltlvdtg igmtkadlin nlgtiaksgt kafmealqag
121 adismigqfg vgfysaylva ekvvvitkhn ddegyawess aggsftvrad hgepigrgtk
181 vilhlkedqt eyleerrvke vvkhsqfig ypitlyleke rekeisddea eeekgekeee
241 dkddeekpki edvgsdeedd sgdkdkkktk kikekyidqe elnktkpiwt rnpdditqee
301 ygefysltn dwedhlavkh fsveggflef allfiprrap fdlfenkkkk nniklyvrrv
361 fimdscdeli peylnfirgv vdsedlplni sremllqgski lkvrknivk kclelfsela
421 edkenykkfy eafsknlklg ihedstnrrr lsellryhts qsgdemtsls eyvsmketq
481 ksiyyitges keqvansafv ervrkrgefv vymtepiday cvqqlkefdg kslvsvtkeg
541 lelpedeeek kkmeeskakf enlcklmkei ldkkvekvti snrlvsspc ivtstygwta
601 nmerimkaqa lrdnstmgym makkhleinp dhpivetlrq kaeadkndka vkdlvllfe
661 tallssgfsl edpqthsnri yrmiklglgi dedevaaeep naavpdeipp legdedasrm
721 eevd
```

HSP 86 (Mouse)  
SEQ ID NO:6

```
1 mpeetqtqdg pmeeeevetf afgaeiaqlm sliintfysn keiflrelis nssdaldkir
61 yesltdpskl dsgkelhinl ipskqdrtilt ivdtgigmtk adlinnlgti aksgkafme
121 alqagadism igqfgvgfys aylvaekvtv itkhnndegy awessaggsf tvrttdtgepm
181 grgtkvilhl kedqteylee rrikeivkhh sqfigypitl fvekerdkev sddeaeekke
241 keeekekeek esddkpeied vgsdeeeek kdgdkkkkkk ikeyidqee lnktkpiwtr
301 npdditneey gefyksltn dwedhlavkh fsveggflef llfvprrapf dlfenrkkkn
361 niklyvrrvf imdnceelip eynlfirgv dsedlplnis remllqgskil kvirknlvkk
421 clelftelae dkenykkfy qfsknklgi hedsqnrkkl sellryytsa sgdemvslkd
481 yctrmkcnqk hiyfitgetk dqvansafve rlrkhglevi ymiepiday cvqqlkefegk
541 tlsvvtkegl elpedeeek kqeeektkfe nlckimkdil ekkvekvvvs nrlvtspcci
601 vtstygwtan merimkaqa lrdnstmgyma akkhleinpd hsiietlrq aeadkndksv
661 kdlvillyet allssgfsle dpqthanriy rmiklglgid eddptvddts aavteemopl
721 egddtsrme evd
```

12/25

FIG. 10



3/25

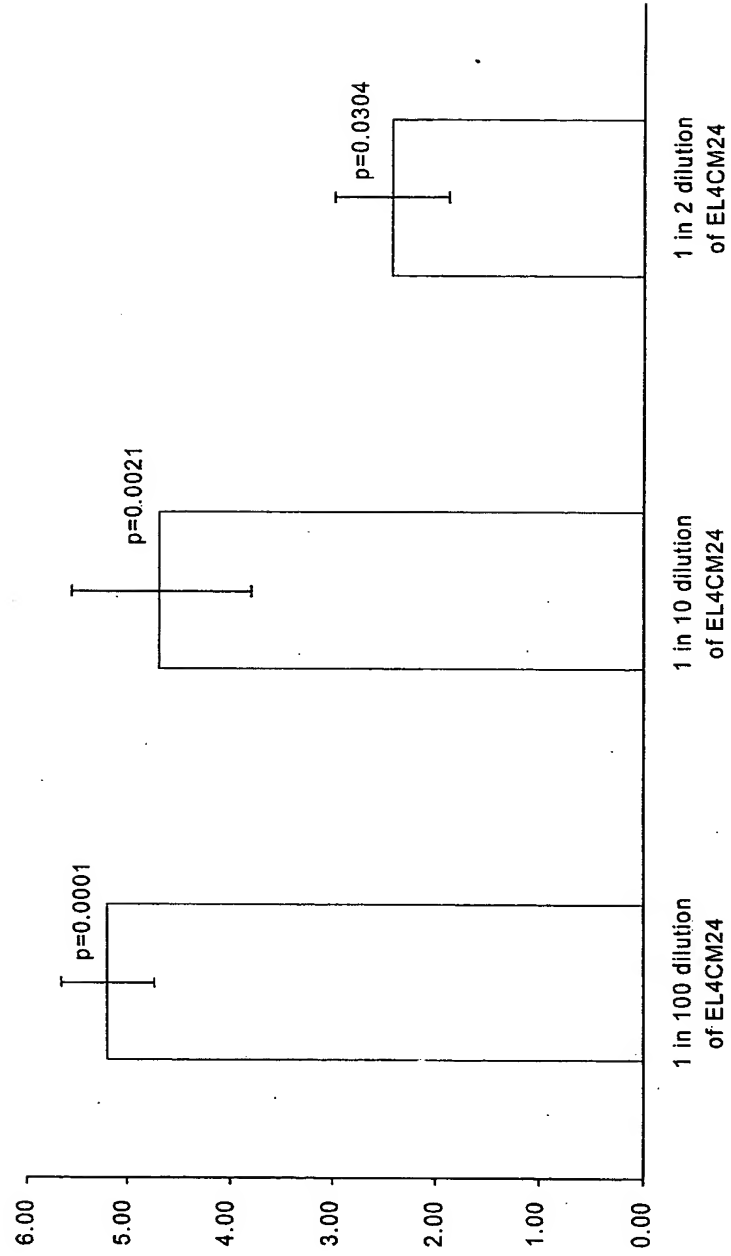
HSP 86, HSP 60 (Human)  
SEQ ID NO:7

```
1 mlrlptvfrq mrpvsirvlap hltrayakdv kfgadaralm lqgvdllda vavtmgpkgr
61 tviieqswgs pkvtkdgvttv aksidlkdky knigaklvqd vanntneeag dgtttatvla
121 rsiakegfek iskganpvei rrgvmlavda viaelkkqsk pvttpeeiaq vatisangdk
181 eigniidam kkvgrkgvit vkdgkltnde leiiegmkd rgyispyfin tskgqkcefq
241 dayvllsekk issiqsiypa leianahrkp lviiadvdg ealstlvlnr lkvglqvav
301 kapgfgdnrk nqlkdmaiat ggavfgeegl tlnledvqph dlkvgeviv tkddamlkg
361 kgdkaqiekr iqeiieqldv ttseyekekl nerlaklsdg vavlkvggts dvevnekkdr
421 vtdalnatra aveegivlgg gcallrcipa ldsltpaned qkigieiikr tlkipamtia
481 knagvegsli vekimqssse vgydamagdf vnmvekgiid ptkvvtall daagvasilt
541 taevvvteip keekdpngma mggmgggmgg gmf
```

L-plastin (Human)  
SEQ ID NO:8

```
1 margsvsdee mmelreafak vtdgngyis fnelndlfka acplpggyrv reitenlmat
61 gdldqdgri fdefikifhg lkstdvaktf rkainkkegi caiggtsegs svgtqhsyse
121 eekyafvnwi nkalendpdc rhvipmpnt ndlfnavgdg ivlckminls vpdtiderti
181 nkkltpfti qenlnlals asaigchvvn igaedlkegk pylvlglwq vikiglfadi
241 elsrnealia llregesled lmklspeell lrwanyhlen agcnkignfs tdikdskayy
301 hileqvapkg deegvpavvi dmsglrekdd iqraecmlqg aerlgcrqfv tatdvrgnp
361 klnlafianl fnrypalkhp enqdidwgal egetreertf rnwmmslgvn prvnhllysd
421 sdalvifgly ekikvpvdown rvnkppypkl ggnmkklenc nyavelgknq akfslvgigg
481 qdlnegnrtl tlaliwqlmr rytlnileei gggqkvnddi ivnwvnetlr eaeksssis
541 fkdpkistsl pvldlidaig pgsinydllk tenlnddekl nnakyaisma rkigarvyal
601 pedlvevnpk mvmtvfacim gkgmkrv
```

**FIG. 2**  
EL4 Conditioned Media Repels T cell *in vitro*



**FIG. 3**  
Heat Inactivation and Proteinase K Digestion of EL4CM24

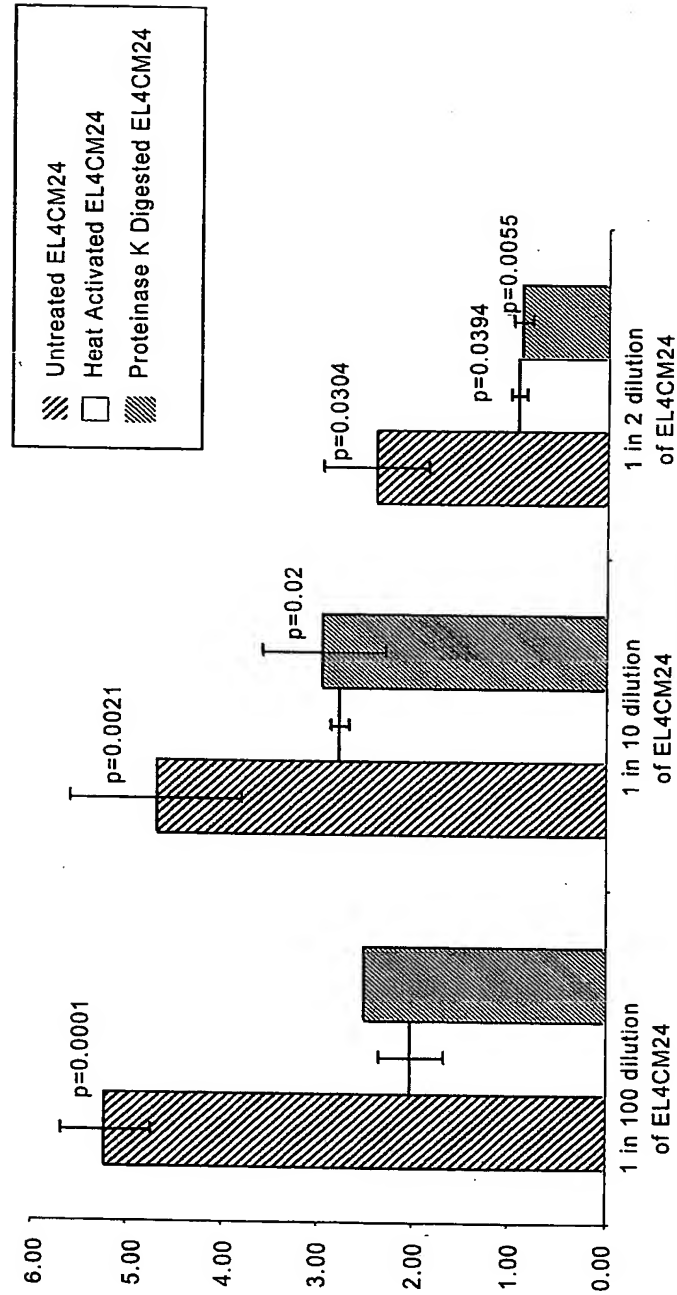
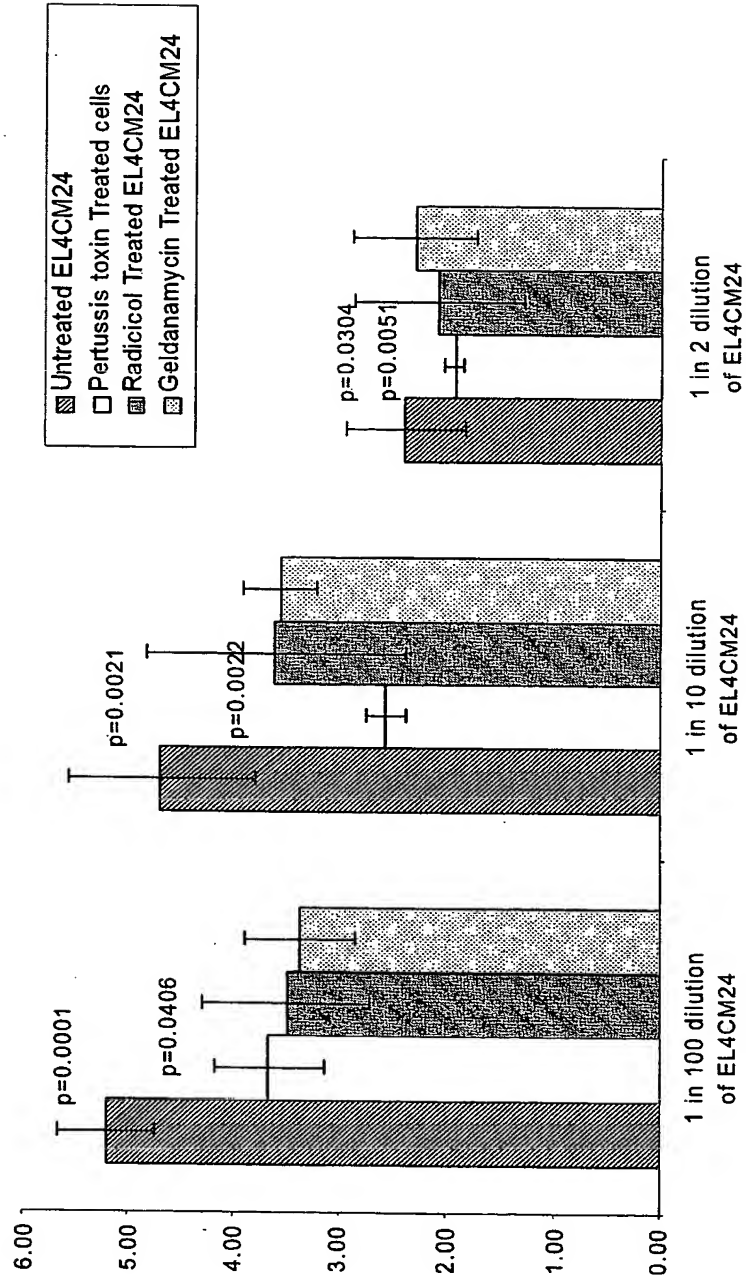


FIG. 4

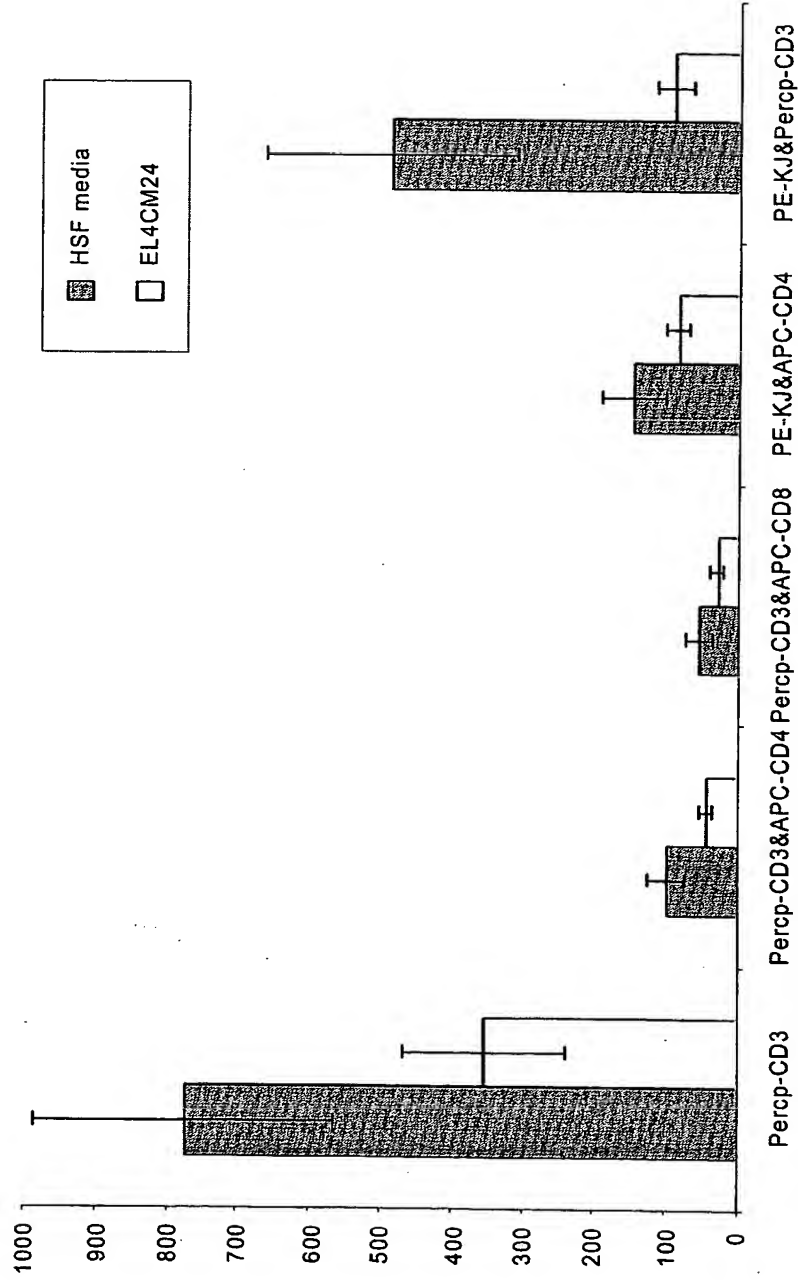
Use of Specific Inhibitors



7/25

FIG. 5

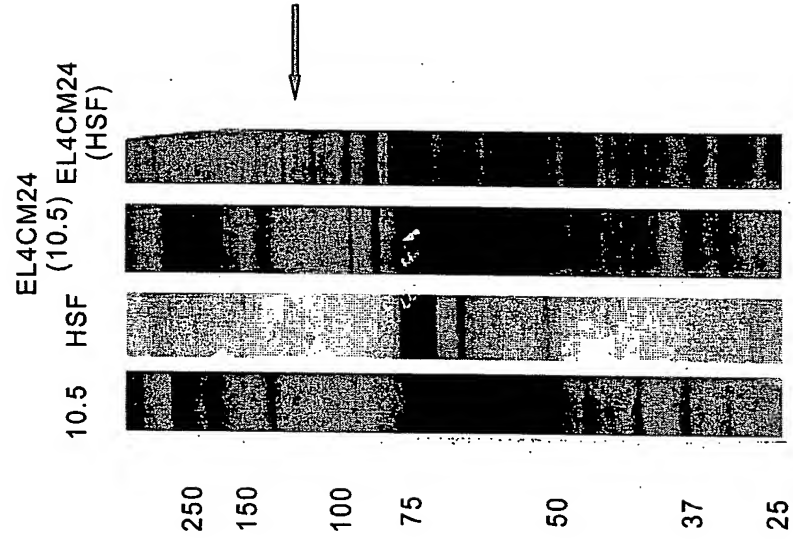
EL4 Conditioned Media Repels T cells *in vivo*





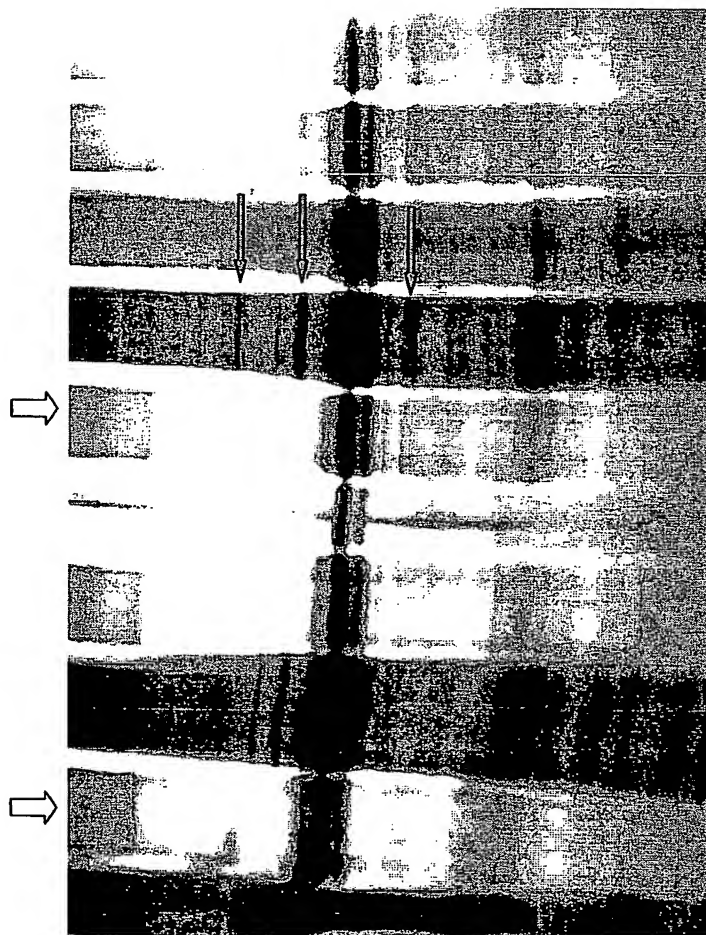
8/25

**FIG. 6**  
EL4CM24 SDS PAGE



9/25

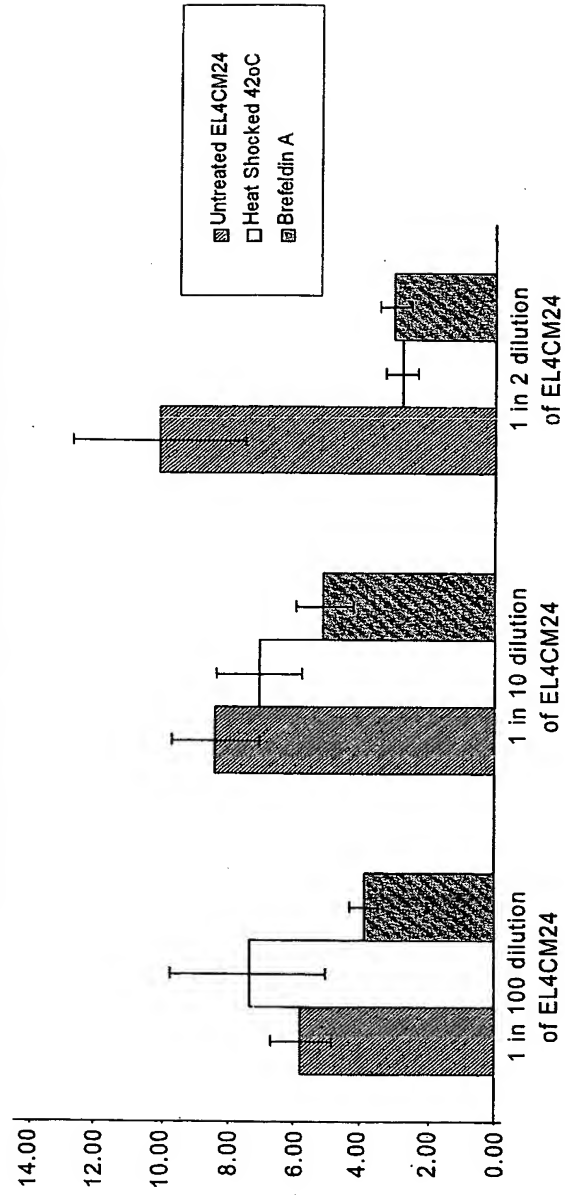
**FIG. 7**  
Ion Exchange Chromatography



10/25

FIG. 8

Heat Shock of EL4 @ 42°C and Brefeldin A treatment of EL4



11/25

FIG. 9

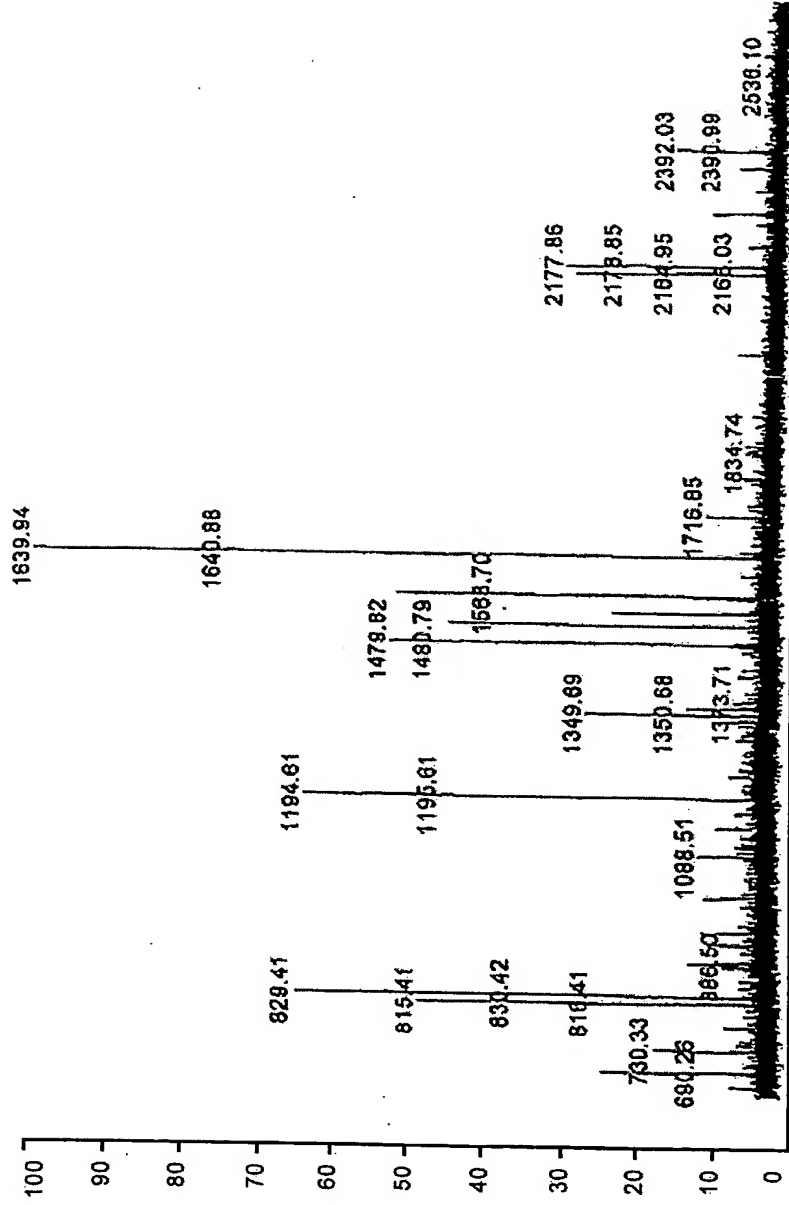
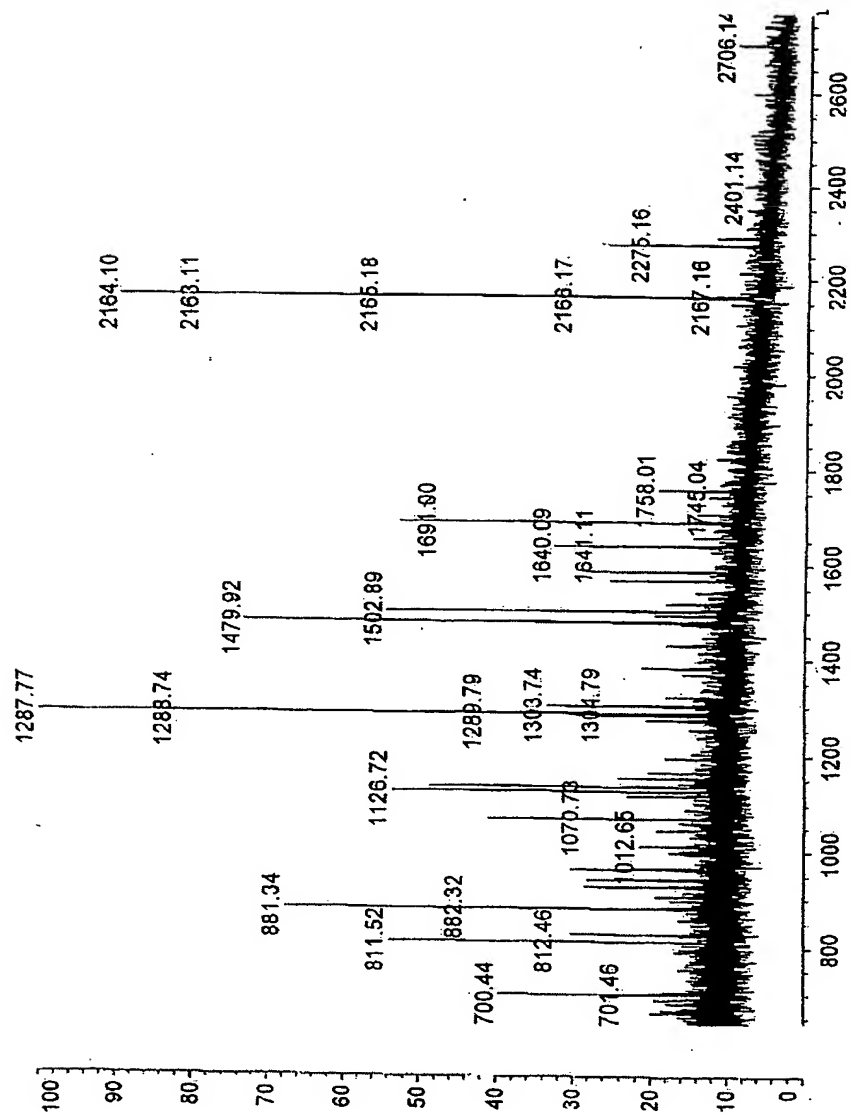


FIG. 11



14/25

FIG. 12A

## MS-Fit Search Results

Press stop on your browser if you wish to abort this MS-Fit search prematurely.

Sample ID (comment): Magic Bullet digest

Databasc searched: NCBI nr.121002

Molecular weight search (1000 - 100000 Da) selects 1195692 entries.

Full pI range: 1247039 entries.

Species search ( MAMMALS ) selects 197947 entries.

Combined molecular weight, pI and species searches select 186900 entries.

MS-Fit search selects 407 entries (results displayed for top 4 matches).

Considered modifications: | Peptide N-terminal Gln to pyroGlu | Oxidation of M | Protein N-terminus Acetylated |

| Min. #<br>Peptides<br>to Match | Peptide<br>Mass<br>Tolerance<br>(+/-)<br>ppm | Peptide<br>Masses<br>are<br>monoisotopic | Digest<br>Used<br>Trypsin | Max. #<br>Missed<br>Cleavages<br>1 | Cysteines<br>Modified<br>by<br>acrylamide | Peptide<br>N terminus<br>Hydrogen (H) | Peptide<br>C terminus<br>Free Acid (O H) | Input #<br>Peptide<br>Masses<br>15 |
|--------------------------------|--|--|---------------------------|------------------------------------|---|---------------------------------------|--|------------------------------------|
| 4                              | 150.000                                      |  |                           |                                    |   |                                       |  |                                    |

## Result Summary

| Rank     | MOWSE<br>Score | # (%)<br>Masses<br>Matched | Protein<br>MW (Da)/pI | Species        | NCBI nr.121002<br>Accession # | Protein Name                   |
|----------|----------------|----------------------------|-----------------------|----------------|-------------------------------|--------------------------------|
| <u>1</u> | 7.02e+003      | 8/15 (53%)                 | 81963.2 / 4.99        | EQUUS CABALLUS | <u>20177936</u>               | heat shock protein 90 beta     |
| <u>2</u> | 6.91e+003      | 8/15 (53%)                 | 83264.6 / 4.97        | HOMO SAPIENS   | <u>20149594</u>               | Unknown (protein for MGC:1138) |
| <u>3</u> | 6.79e+003      | 8/15 (53%)                 | 84843.9 / 5.26        | HOMO SAPIENS   | <u>11277141</u>               | hypothetical protein           |
| <u>4</u> | 2.25e+003      | 7/15 (46%)                 | 83316.8 / 5.06        | RATTUS SP.     | <u>1346320</u>                | heat shock protein 90; hsp90   |

## Detailed Results

1. 8/15 matches (53%). 81963.2 Da, pI = 4.99. Acc. # 20177936. EQUUS CABALLUS. heat shock protein 90 beta .

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 689.3000         | 689.3946                   | -137.2410    | 570   | 575 | (K)VTISNR(L)                                  |               |
| 829.4100         | 829.5300                   | -144.6415    | 323   | 329 | (R)ALLFIPR(R)                                 |               |
| 891.3500         | 891.4252                   | -84.4094     | 421   | 427 | (K)FYEAFFSK(N)                                |               |
| 1194.6100        | 1194.6483                  | -32.0277     | 65    | 74  | (K)IDIIPNPOER(T)                              |               |
| 1348.6900        | 1348.6650                  | 18.5557      | 312   | 322 | (K)HFSVEGOLEFR(A)                             |               |
| 1513.7800        | 1513.7862                  | -4.1036      | 371   | 384 | (R)GVVDSEDLPLNISR(E)                          |               |
| 2176.8600        | 2176.9457                  | -39.3681     | 449   | 467 | (R)YHTSQSGDEMTSLSEYVSR(M)                     |               |
| 2390.9900        | 2391.1832                  | -80.8096     | 474   | 494 | (K)SIYYITGESKEOVANSAFVER(V)                   |               |

7 unmatched masses: 730.3300 815.4100 1479.8200 1537.7700 1567.7000 1639.9400 1715.8900

The matched peptides cover 13% (95/713 AA's) of the protein.

Coverage Map for This Hit (MS-Digest index #): 1205701

15/25

FIG. 12B

2. 8/15 matches (53%). 83264.6 Da, pI = 4.97. Acc. # 20149594. HOMO SAPIENS. Unknown (protein for MGC:1138).

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 689.3000         | 689.3946                   | -137.2410    | 578   | 583 | (K)VTISNR(L)                                  |               |
| 829.4100         | 829.5300                   | -144.6415    | 331   | 337 | (R)ALLFIPR(R)                                 |               |
| 891.3500         | 891.4252                   | -84.4094     | 429   | 435 | (K)FYEAFSK(N)                                 |               |
| 1194.6100        | 1194.6483                  | -32.0277     | 73    | 82  | (K)IDHPNPOER(T)                               |               |
| 1348.6900        | 1348.6650                  | 18.5557      | 320   | 330 | (K)HFSVEGOLEFR(A)                             |               |
| 1513.7800        | 1513.7862                  | -4.1036      | 379   | 392 | (R)GVYDSEDLPLNISR(E)                          |               |
| 2176.8600        | 2176.9457                  | -39.3681     | 457   | 475 | (R)YHTSOSGDEMTSLSEYVSR(M)                     |               |
| 2390.9900        | 2391.1832                  | -80.8096     | 482   | 502 | (K)SIYYITGESKEQVANSAFYER(V)                   |               |

7 unmatched masses: 730.3300 815.4100 1479.8200 1537.7700 1567.7000 1639.9400 1715.8900

The matched peptides cover 13% (95/724 AA's) of the protein.  
Coverage Map for This Hit (MS-Digest index #): [137455](#)

3. 8/15 matches (53%). 84843.9 Da, pI = 5.26. Acc. # 11277141. HOMO SAPIENS. hypothetical protein.

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 689.3000         | 689.3946                   | -137.2410    | 578   | 583 | (K)VTISNR(L)                                  |               |
| 829.4100         | 829.5300                   | -144.6415    | 331   | 337 | (R)ALLFIPR(R)                                 |               |
| 891.3500         | 891.4252                   | -84.4094     | 429   | 435 | (K)FYEAFSK(N)                                 |               |
| 1194.6100        | 1194.6483                  | -32.0277     | 73    | 82  | (K)IDHPNPOER(T)                               |               |
| 1348.6900        | 1348.6650                  | 18.5557      | 320   | 330 | (K)HESVEGOLEFR(A)                             |               |
| 1513.7800        | 1513.7862                  | -4.1036      | 379   | 392 | (R)GVYDSEDLPLNISR(E)                          |               |
| 2176.8600        | 2176.9457                  | -39.3681     | 457   | 475 | (R)YHTSOSGDEMTSLSEYVSR(M)                     |               |
| 2390.9900        | 2391.1832                  | -80.8096     | 482   | 502 | (K)SIYYITGESKEQVANSAFYER(V)                   |               |

7 unmatched masses: 730.3300 815.4100 1479.8200 1537.7700 1567.7000 1639.9400 1715.8900

The matched peptides cover 12% (95/737 AA's) of the protein.  
Coverage Map for This Hit (MS-Digest index #): [1101236](#)

4. 7/15 matches (46%). 83316.8 Da, pI = 5.06. Acc. # 1346320. RATTUS SP.. heat shock protein 90: hsp90.

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 689.3000         | 689.3946                   | -137.2410    | 578   | 583 | (K)VTISNR(L)                                  |               |
| 829.4100         | 829.5300                   | -144.6415    | 331   | 337 | (R)ALLFIPR(R)                                 |               |
| 891.3500         | 891.4252                   | -84.4094     | 429   | 435 | (K)FYEAFSK(N)                                 |               |
| 1348.6900        | 1348.6650                  | 18.5557      | 320   | 330 | (K)HESVEGOLEFR(A)                             |               |
| 1513.7800        | 1513.7862                  | -4.1036      | 379   | 392 | (R)GVYDSEDLPLNISR(E)                          |               |
| 2176.8600        | 2176.9457                  | -39.3681     | 457   | 475 | (R)YHTSOSGDEMTSLSEYVSR(M)                     |               |
| 2390.9900        | 2391.1832                  | -80.8096     | 482   | 502 | (K)SIYYITGESKEQVANSAFYER(V)                   |               |

8 unmatched masses: 730.3300 815.4100 1194.6100 1479.8200 1537.7700 1567.7000 1639.9400 1715.8900

## MS-Tag Search Results

Press stop on your browser if you wish to abort this MS-Tag search prematurely.

Sample ID (comment): Apo A-1 1040 AKPVLEDLR

Database searched: NCBI nr.121002

Full Molecular Weight range: 1247039 entries.

Full pI range: 1247039 entries.

Species search ( MAMMALS ) selects 197947 entries.

Number of sequences passing through parent mass filter: 4253

MS-Tag search selects 7 entries (results displayed for top 3 matches).

Parent mass: 1194.6100 (+/- 0.2000 Da)

Fragment ions used in search: 175.31, 212.00, 229.20, 342.62, 355.43, 512.43, 529.50, 626.31, 723.44, 740.58, 966.73 (+/- 0.50 Da)

Ion Types Considered: a b y o h l

| Search<br>Mode<br>Identify | Max. #<br>Unmatched<br>Ions | Peptide Masses<br>are<br>monoisotopic | Digest<br>Used<br>Trypsin | Max. # Missed<br>Cleavages | Cysteines<br>Modified by<br>acrylamide | Peptide<br>N terminus<br>Hydrogen (H) | Peptide<br>C terminus<br>Free Acid (O H) |
|----------------------------|-----------------------------|---------------------------------------|---------------------------|----------------------------|--|---------------------------------------|--|
|                            | 1                           |                                       |                           | 1                          |  |                                       |  |

## Result Summary

| Rank | #<br>Unmatched<br>Ions | Sequence        | MH <sup>+</sup><br>Calculated<br>(Da) | MH <sup>+</sup><br>Error<br>(Da) | Protein<br>MW (Da)/pI | Species      | NCBI nr.121002<br>Accession # | Protein Name   |
|------|------------------------|-----------------|---------------------------------------|----------------------------------|-----------------------|--------------|-------------------------------|--|
| 1    | 0/11                   | (K)IDHPNPOER(T) | 1194.6483                             | -0.0383                          | 83325.7 / 4.97        | MOUSE        | <a href="#">123681</a>        | Heat shock protein HSP 90-beta (HSP 94)<br>(Tumor specific transplantation 84 kDa antigen)<br>(TSTA) |
| 1    | 0/11                   | (K)IDHPNPOER(T) | 1194.6483                             | -0.0383                          | 83361.1 / 5.03        | MUS MUSCULUS | <a href="#">6680305</a>       | heat shock protein, 84 kDa 1   |
| 1    | 0/11                   | (K)IDHPNPOER(T) | 1194.6483                             | -0.0383                          | 14066.4 / 4.64        | HOMO SAPIENS | <a href="#">2351110</a>       | heat shock protein beta  |

FIG. 12C

| Rank               | #<br>Unmatched<br>Ions | Sequence                | MH <sup>+</sup><br>Calculated<br>(Da) | MH <sup>+</sup><br>Error<br>(Da) | Protein<br>MW (Da)/pI | Species      | NCBI.Xr.121002<br>Accession #   | MS-Digest<br>Index # | Protein Name   |                                 |                |                |
|--------------------|------------------------|-------------------------|---------------------------------------|----------------------------------|-----------------------|--------------|---------------------------------|----------------------|--|---------------------------------|----------------|----------------|
| 1                  | 9/11                   | (K) <u>IDILPNQER(T)</u> | 1194.6493                             | -0.0383                          | 83325.7 / 4.97        | MOUSE        | <u>123681</u>                   | <u>318176</u>        | Heat shock protein HSP 90-beta (HSP 84) (Tumor specific transplantation 84 kDa antigen) (TSTA) |                                 |                |                |
| 1                  | 0/11                   | (K) <u>IDILPNQER(T)</u> | 1194.6483                             | -0.0383                          | 83361.1 / 5.03        | MUS MUSCULUS | <u>6680305</u>                  | <u>583990</u>        | heat shock protein, 84 kDa I   |                                 |                |                |
| <hr/>              |                        |                         |                                       |                                  |                       |              |                                 |                      |  |                                 |                |                |
| Fragment-Ion (m/z) |                        | 175.31                  | 212.00                                | 229.20                           | 342.62                | 355.43       | 512.43                          | 529.50               | 626.31   | 723.44                          | 740.58         | 966.73         |
| Ion-type           |                        | y <sub>1</sub>          | NP                                    | b <sub>2</sub>                   | b <sub>3</sub>        | PQE          | y <sub>4</sub> -NH <sub>3</sub> | y <sub>4</sub>       | y <sub>5</sub> -NH <sub>3</sub>  | y <sub>6</sub> -NH <sub>3</sub> | y <sub>6</sub> | y <sub>8</sub> |
| Delta Da           |                        | 0.19                    | -0.10                                 | 0.03<br>D1                       | 0.42<br>DIL           | 0.27         | 0.18                            | 0.23                 | 0.02   | 0.10                            | 0.21           | 0.19           |
|                    |                        |                         |                                       | 0.08                             | 0.42                  |              |                                 |                      |  |                                 |                |                |
| 1                  | 0/11                   | (K) <u>IDILPNQER(T)</u> | 1194.6483                             | -0.0383                          | 14066.4 / 4.64        | HOMO SAPIENS | <u>2351110</u>                  | <u>587097</u>        | heat shock protein beta  |                                 |                |                |
| Fragment-Ion (m/z) |                        | 175.31                  | 212.00                                | 229.20                           | 342.62                | 355.43       | 512.43                          | 529.50               | 626.31   | 723.44                          | 740.58         | 966.73         |
| Ion-type           |                        | y <sub>1</sub>          | NP                                    | b <sub>2</sub>                   | b <sub>3</sub>        | PQE          | y <sub>4</sub> -NH <sub>3</sub> | y <sub>4</sub>       | y <sub>5</sub> -NH <sub>3</sub>  | y <sub>6</sub> -NH <sub>3</sub> | y <sub>6</sub> | y <sub>8</sub> |
| Delta Da           |                        | 0.19                    | -0.10                                 | 0.03<br>D1                       | 0.42<br>DII           | 0.27         | 0.18                            | 0.23                 | 0.02   | 0.10                            | 0.21           | 0.19           |
|                    |                        |                         |                                       | 0.08                             | 0.42                  |              |                                 |                      |  |                                 |                |                |

### MS-Tag Search Results

Press stop on your browser if you wish to abort this MS-Tag search prematurely.

Sample ID (comment): Apo A-1 1040 AKPVLEDLR

Database searched: NCBI nr.121002

Molecular weight search (1000 - 100000 Da) selects 1195692 entries.

Full pl range: 1247039 entries.

Species search ( MAMMALS ) selects 197947 entries:

Combined molecular weight, pI and species searches select 186900 entries.

Number of sequences passing through parent mass filter: 4989

MS-Tag search selects 18 entries (results displayed for top 3 matches).

Parent mass: 815.4100 (+/- 0.2000 Da)

Fragment Ions used in search: 185.26, 255.27, 272.34, 298.32, 354.45, 371.53, 417.39, 445.25, 518.35 (+/- 0.50 Da)

Ion Types Considered: a b B y n h I

| Search<br>Mode<br>identity | Max. #<br>Unmatched<br>Ions<br>1 | Peptide Masses<br>are<br>monoisotopic | Digest<br>Used<br>Trypsin | Max. # Missed<br>Cleavages<br>1 | Cysteines<br>Modified by<br>acrylamide | Peptide<br>N terminus<br>Hydrogen (H) | Peptide<br>C terminus<br>Free Acid (O H) |
|----------------------------|----------------------------------|---------------------------------------|---------------------------|---------------------------------|--|---------------------------------------|--|
|----------------------------|----------------------------------|---------------------------------------|---------------------------|---------------------------------|--|---------------------------------------|--|

### Result Summary

| Rank | # Unmatched Ions | Sequence      | MH <sup>+</sup> Calculated (Da) | MH <sup>+</sup> Error (Da) | Protein MW (Da)/pI | Species      | NCBI Ref. 121002 Accession # | Protein Name                         |
|------|------------------|---------------|---------------------------------|----------------------------|--------------------|--------------|------------------------------|--------------------------------------|
| 1    | 0/9              | (R)ALLFVPR(R) | 815.5143                        | -0.1043                    | 75541.0 / 5.28     | MUS MUSCULUS | <u>70887565</u>              | similar to heat shock protein 86     |
| 1    | 0/9              | (R)ALLFVPR(R) | 815.5143                        | -0.1043                    | 84674.2 / 4.94     | HOMO SAPIENS | <u>123678</u>                | 90 kDa heat-shock protein (AA 1-732) |
| 1    | 0/9              | (K)ALLFVPR(R) | 815.5143                        | -0.1043                    | 57668.0 / 6.38     | HOMO SAPIENS | <u>12804541</u>              | hypothetical protein LOC63929        |

| Rank               | # Unmatched Ions | Sequence      | MH <sup>+</sup> Calculated (Da) | MH <sup>-</sup> Error (Da)                   | Protein MW (Da)/pI | Species        | NCBI nr.121002 Accession #                   | MS-Digest Index # | Protein Name                         |                |                |
|--------------------|------------------|---------------|---------------------------------|--|--------------------|----------------|--|-------------------|--------------------------------------|----------------|----------------|
| 1                  | 0/9              | (R)ALLFVPR(R) | 815.5143                        | -0.1043                                      | 75541.0 / 5.28     | MUS MUSCULUS   | <u>20882565</u>                              | <u>613811</u>     | similar to heat shock protein 86     |                |                |
| 1                  | 0/9              | (R)ALLFVPR(R) | 815.5143                        | -0.1043                                      | 84674.2 / 4.94     | HOMO SAPIENS   | <u>123678</u>                                | <u>162860</u>     | 90 kDa heat-shock protein (AA 1-732) |                |                |
| <hr/>              |                  |               |                                 |  |                    |                |  |                   |                                      |                |                |
| Fragment-ion (m/z) |                  |               | 185.26                          | 255.27                                       | 272.34             | 298.32         | 354.45                                       | 371.53            | 417.39                               | 445.25         | 518.35         |
| Ion-type           |                  |               | b <sub>2</sub>                  | y <sub>2</sub> -NH <sub>3</sub> <sup>+</sup> | y <sub>2</sub>     | b <sub>3</sub> | y <sub>3</sub> -NH <sub>3</sub> <sup>+</sup> | y <sub>3</sub>    | a <sub>4</sub>                       | b <sub>4</sub> | y <sub>4</sub> |
| Delta Da           |                  |               | 0.13                            | 0.12   | 0.17               | 0.11           | 0.24   | 0.29              | 0.10                                 | -0.03          | 0.04           |
| <hr/>              |                  |               |                                 |  |                    |                |  |                   |                                      |                |                |
| f                  | 0/9              | (K)AILFVPR(R) | 815.5143                        | -0.1043                                      | 57068.0 / 6.38     | HOMO SAPIENS   | <u>12804541</u>                              | <u>171353</u>     | hypothetical protein LOC63929        |                |                |
| <hr/>              |                  |               |                                 |  |                    |                |  |                   |                                      |                |                |
| Fragment-ion (m/z) |                  |               | 185.26                          | 255.27                                       | 272.34             | 298.32         | 354.45                                       | 371.53            | 417.39                               | 445.25         | 518.35         |
| Ion-type           |                  |               | b <sub>2</sub>                  | y <sub>2</sub> -NH <sub>3</sub> <sup>+</sup> | y <sub>2</sub>     | b <sub>3</sub> | y <sub>3</sub> -NH <sub>3</sub> <sup>+</sup> | y <sub>3</sub>    | a <sub>4</sub>                       | b <sub>4</sub> | y <sub>4</sub> |
| Delta Da           |                  |               | 0.13                            | 0.12   | 0.17               | 0.11           | 0.24   | 0.29              | 0.10                                 | -0.03          | 0.04           |



17/25

FIG. 13A

## MS-Fit Search Results

Press stop on your browser if you wish to abort this MS-Fit search prematurely.

Sample ID (comment): Magic Bullet digest

Database searched: NCBI nr.51403

Molecular weight search (1000 - 200000 Da) selects 1421445 entries.

Full pI range: 1432416 entries.

Species search ( HUMAN RODENT ) selects 224838 entries.

Combined molecular weight, pI and species searches select 222557 entries.

MS-Fit search selects 5 entries (results displayed for top 3 matches).

Considered modifications: | Peptide N-terminal Gln to pyroGlu | Oxidation of M | Protein N-terminus Acetylated |

| Min. #<br>Peptides<br>to Match | Peptide<br>Mass<br>Tolerance<br>(+/-)<br>ppm | Peptide<br>Masses<br>are<br>monoisotopic | Digest<br>Used<br>Trypsin | Max. #<br>Missed<br>Cleavages | Cysteines<br>Modified<br>by<br>acrylamide | Peptide<br>N terminus<br>Hydrogen (H) | Peptide<br>C terminus<br>Free Acid (O H) | Input #<br>Peptide<br>Masses |
|--------------------------------|--|--|---------------------------|-------------------------------|---|---------------------------------------|--|------------------------------|
| 7                              | 150.000                                      |  |                           | 1                             |   |                                       |  | 13                           |

## Result Summary

| Rank | MOWSE<br>Score | # (%)<br>Masses<br>Matched | Protein<br>MW (Da)/pI | Species           | NCBI nr.51403<br>Accession # | Protein Name                       |
|------|----------------|----------------------------|-----------------------|-------------------|------------------------------|------------------------------------|
| 1    | 1.81e+003      | 7/13 (53%)                 | 94057.0 / 5.13        | RATTUS NORVEGICUS | <a href="#">24025637</a>     | ischemia responsive 94 kDa protein |
| 2    | 449            | 7/13 (53%)                 | 94081.1 / 5.13        | MUS MUSCULUS      | <a href="#">13277753</a>     | heat shock protein 4               |
| 2    | 449            | 7/13 (53%)                 | 94133.1 / 5.15        | MUS MUSCULUS      | <a href="#">6680301</a>      | apg-2                              |

## Detailed Results

1. 7/13 matches (53%). 94057.0 Da, pI = 5.13. Acc. # 24025637. RATTUS NORVEGICUS. ischemia responsive 94 kDa protein.

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 798.5500         | 798.4514                   | 123.4893     | 431   | 436 | (K) <a href="#">VLTFYR</a> (K)                |               |
| 949.6100         | 949.5219                   | 92.7425      | 62    | 69  | (K) <a href="#">NTVQGFKR</a> (F)              |               |
| 1321.8500        | 1321.7116                  | 104.7200     | 222   | 234 | (K) <a href="#">VLATAFDITLGG</a> (K)          |               |
| 1402.7800        | 1402.6313                  | 106.0213     | 619   | 629 | (K) <a href="#">NAVEEYYEMR</a> (D)            |               |
| 1495.8400        | 1495.7029                  | 91.6785      | 20    | 33  | (R) <a href="#">AGGIETIANEYSDR</a> (C)        |               |
| 1736.0700        | 1735.9271                  | 82.3407      | 391   | 405 | (R) <a href="#">EFSITDVVPYFISLR</a> (W)       |               |
| 1953.0400        | 1952.8336                  | 105.6759     | 406   | 422 | (R) <a href="#">WNSPAEEGSSDCEVFPK</a> (N)     |               |

6 unmatched masses: 915.6000 917.3600 1305.8400 1478.8800 1587.9500 1624.0500

The matched peptides cover 10% (84/840 AA's) of the protein.  
Coverage Map for This Hit (MS-Digest Index #): [787619](#)

2. 7/13 matches (53%). 94081.1 Da, pI = 5.13. Acc. # 13277753. MUS MUSCULUS. heat shock protein 4.

18/25

FIG. 13B

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 798.5500         | 798.4514                   | 123.4893     | 431   | 436 | (K) <u>VLTFYR</u> (K)                         |               |
| 949.6100         | 949.5219                   | 92.7425      | 62    | 69  | (K) <u>NTVOGFKR</u> (F)                       |               |
| 1305.8400        | 1305.7418                  | 75.1849      | 670   | 680 | (K) <u>QVYVDKLAELK</u> (S)                    |               |
| 1321.8500        | 1321.7116                  | 104.7200     | 222   | 234 | (K) <u>VLATAFDITLGGR</u> (K)                  |               |
| 1402.7800        | 1402.6313                  | 106.0213     | 620   | 630 | (K) <u>NAVEEYVYEMR</u> (D)                    |               |
| 1495.8400        | 1495.7029                  | 91.6785      | 20    | 33  | (R) <u>AGGIETIANEYSDR</u> (C)                 |               |
| 1736.0700        | 1735.9271                  | 82.3407      | 391   | 405 | (R) <u>EFSITDVVPYPISLR</u> (W)                |               |

6 unmatched masses: 915.6000 917.3600 1478.8800 1587.9500 1624.0500 1953.0400

The matched peptides cover 9% (78/841 AA's) of the protein.  
Coverage Map for This Hit (MS-Digest index #): 202745

2. 7/13 matches (53%). 94133.1 Da, pI .. 5.15, Acc. # 6680301, MUS MUSCULUS, apg-2 .

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 798.5500         | 798.4514                   | 123.4893     | 431   | 436 | (K) <u>VLTFYR</u> (K)                         |               |
| 949.6100         | 949.5219                   | 92.7425      | 62    | 69  | (K) <u>NTVOGFKR</u> (F)                       |               |
| 1305.8400        | 1305.7418                  | 75.1849      | 670   | 680 | (K) <u>QVYVDKLAELK</u> (S)                    |               |
| 1321.8500        | 1321.7116                  | 104.7200     | 222   | 234 | (K) <u>VLATAFDITLGGR</u> (K)                  |               |
| 1402.7800        | 1402.6313                  | 106.0213     | 620   | 630 | (K) <u>NAVEEYVYEMR</u> (D)                    |               |
| 1495.8400        | 1495.7029                  | 91.6785      | 20    | 33  | (R) <u>AGGIETIANEYSDR</u> (C)                 |               |
| 1736.0700        | 1735.9271                  | 82.3407      | 391   | 405 | (R) <u>EFSITDVVPYPISLR</u> (W)                |               |

6 unmatched masses: 915.6000 917.3600 1478.8800 1587.9500 1624.0500 1953.0400

The matched peptides cover 9% (78/841 AA's) of the protein.  
Coverage Map for This Hit (MS-Digest index #): 1179227

19/25

FIG. 14A

## MS-Fit Search Results

Press stop on your browser if you wish to abort this MS-Fit search prematurely.

Sample ID (comment): Magic Bullet digest

Database searched: NCBI nr.51403

Molecular weight search (1000 - 100000 Da) selects 1372760 entries.

Full pI range: 1432416 entries.

Species search ( HUMAN RODENT ) selects 224838 entries.

Combined molecular weight, pI and species searches select 211465 entries.

MS-Fit search selects 335 entries (results displayed for top 3 matches).

Considered modifications: | Peptide N-terminal Gln to pyroGlu | Oxidation of M | Protein N-terminus Acetylated |

| Min. #<br>Peptides<br>to Match | Peptide<br>Mass<br>Tolerance<br>(+/-)<br>ppm | Peptide<br>Masses<br>are<br>monoisotopic | Digest<br>Used<br>Trypsin | Max. #<br>Missed<br>Cleavages | Cysteines<br>Modified<br>by<br>acrylamide | Peptide<br>N terminus<br>Hydrogen (H) | Peptide<br>C terminus<br>Free Acid (O H) | Input #<br>Peptide<br>Masses |
|--------------------------------|--|--|---------------------------|-------------------------------|---|---------------------------------------|--|------------------------------|
| 4                              | 150,000                                      |  |                           | 1                             |   |                                       |  | 17                           |

## Result Summary

| Rank | MOWSE<br>Score | # (%)<br>Masses<br>Matched | Protein<br>MW (Da)/pI | Species      | NCBI nr.51403<br>Accession # | Protein Name              |
|------|----------------|----------------------------|-----------------------|--------------|------------------------------|---------------------------|
| 1    | 1.22e+005      | 11/17 (64%)                | 70149.7 / 5.20        | MUS MUSCULUS | <u>29840803</u>              | unnamed protein product   |
| 2    | 1.22e+005      | 11/17 (64%)                | 70163.8 / 5.24        | MUS MUSCULUS | <u>26326929</u>              | unnamed protein product   |
| 3    | 2.66e+004      | 10/17 (58%)                | 70201.8 / 5.28        | MUS MUSCULUS | <u>6679385</u>               | 65-kDa macrophage protein |

## Detailed Results

1. 11/17 matches (64%). 70149.7 Da, pI = 5.20. Acc. # 29840803. MUS MUSCULUS. unnamed protein product .

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 700.4400         | 700.4146                   | 36.2352      | 77    | 82  | (K)VFHGLK(S)                                  |               |
| 811.5200         | 811.4136                   | 131.0872     | 585   | 591 | (K)YAI SMAR(K)                                |               |
| 942.5800         | 942.5413                   | 41.0831      | 442   | 449 | (R)Y NKPPYPK(L)                               |               |
| 1069.7200        | 1069.6257                  | 88.1285      | 264   | 272 | (K)LSPEELL LR(W)                              |               |
| 1126.7200        | 1126.6373                  | 73.4015      | 433   | 441 | (K)IKVPVDWNR(V)                               |               |
| 1135.7100        | 1135.6111                  | 87.0474      | 348   | 357 | (R)QFVTATDVVR(G)                              |               |
| 1287.7700        | 1287.6268                  | 111.2008     | 402   | 412 | (R)NWMNSLG VNPR(V)                            |               |
| 1502.8900        | 1502.7525                  | 91.5194      | 166   | 178 | (K)MINLSVPI TIDER(T)                          |               |
| 1585.9400        | 1585.8477                  | 58.1710      | 597   | 610 | (R)VYALPEDLVEVNPK(M)                          |               |
| 1689.9700        | 1689.8560                  | 67.4478      | 473   | 488 | (K)FSLVGIACQDLNEGNR(T)                        |               |
| 1758.0100        | 1757.8744                  | 77.1528      | 310   | 326 | (K)GDREGIPAVVIDMSCLR(E)                       |               |

6 unmatched masses: 927.5400 964.6000 1478.8800 1479.9200 1567.8600 1640.0900

The matched peptides cover 19% (120/627 AA's) of the protein.

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20/25

FIG. 14B

Coverage Map for This Hit (MS-Digest index #): 372720

2. 11/17 matches (64%). 70163.8 Da, pI = 5.24. Acc. # 26326929. MUS MUSCULUS. unnamed protein product.

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 700.4400         | 700.4146                   | 36.2352      | 77    | 82  | (K)VFHGLK(S)                                  |               |
| 811.5200         | 811.4136                   | 131.0872     | 585   | 591 | (K)YAISMAR(K)                                 |               |
| 942.5800         | 942.5413                   | 41.0831      | 442   | 449 | (R)VNKPPYPK(L)                                |               |
| 1069.7200        | 1069.6257                  | 88.1285      | 264   | 272 | (K)LSPEELLLR(W)                               |               |
| 1126.7200        | 1126.6373                  | 73.4015      | 433   | 441 | (K)IKVPVDWNR(V)                               |               |
| 1135.7100        | 1135.6111                  | 87.0474      | 348   | 357 | (R)QFVTATDVVR(G)                              |               |
| 1287.7700        | 1287.6268                  | 111.2008     | 402   | 412 | (R)NWMNSLGVNFR(V)                             |               |
| 1502.8900        | 1502.7525                  | 91.5194      | 166   | 178 | (K)MINLSVPDTIDER(T)                           |               |
| 1585.9400        | 1585.8477                  | 58.1710      | 597   | 610 | (R)VYALPEDLVEVNPK(M)                          |               |
| 1689.9700        | 1689.8560                  | 67.4478      | 473   | 488 | (K)FSLVGIACODLNEGNR(T)                        |               |
| 1758.0100        | 1757.8744                  | 77.1528      | 310   | 326 | (K)GDEEGIPAVVIDMSGLR(E)                       |               |

6 unmatched masses: 927.5400 964.6000 1478.8800 1479.9200 1567.8600 1640.0900

The matched peptides cover 19% (120/627 AA's) of the protein.

Coverage Map for This Hit (MS-Digest index #): 1174311

3. 10/17 matches (58%). 70201.8 Da, pI = 5.28. Acc. # 6679385. MUS MUSCULUS. 65-kDa macrophage protein.

| m/z<br>submitted | MH <sup>+</sup><br>matched | Delta<br>ppm | start | end | Peptide Sequence<br>(Click for Fragment Ions) | Modifications |
|------------------|----------------------------|--------------|-------|-----|---|---------------|
| 700.4400         | 700.4146                   | 36.2352      | 77    | 82  | (K)VFHGLK(T)                                  |               |
| 811.5200         | 811.4136                   | 131.0872     | 585   | 591 | (K)YAISMAR(K)                                 |               |
| 942.5800         | 942.5413                   | 41.0831      | 442   | 449 | (R)VNKPPYPK(L)                                |               |
| 1069.7200        | 1069.6257                  | 88.1285      | 264   | 272 | (K)LSPEELLLR(W)                               |               |
| 1126.7200        | 1126.6373                  | 73.4015      | 433   | 441 | (K)IKVPVDWNR(V)                               |               |
| 1135.7100        | 1135.6111                  | 87.0474      | 348   | 357 | (R)QFVTATDVVR(G)                              |               |
| 1287.7700        | 1287.6268                  | 111.2008     | 402   | 412 | (R)NWMNSLGVNFR(V)                             |               |
| 1502.8900        | 1502.7525                  | 91.5194      | 166   | 178 | (K)MINLSVPDTIDER(T)                           |               |
| 1585.9400        | 1585.8477                  | 58.1710      | 597   | 610 | (R)VYALPEDLVEVNPK(M)                          |               |
| 1758.0100        | 1757.8744                  | 77.1528      | 310   | 326 | (K)GDEEGIPAVVIDMSGLR(E)                       |               |

7 unmatched masses: 927.5400 964.6000 1478.8800 1479.9200 1567.8600 1640.0900 1689.9700

The matched peptides cover 16% (104/627 AA's) of the protein.

Coverage Map for This Hit (MS-Digest index #): 746520

FIG. 14C

## MS-Tag Search Results

Press stop on your browser if you wish to abort this MS-Tag search prematurely.

Sample ID (comment): Apo A-1 1040 AKPVLEDLR

Database searched: NCBIInr.51403

Molecular weight search (1000 - 200000 Da) selects 1421445 entries.

Full pI range: 1432416 entries.

Species search ( HUMAN RODENT ) selects 224838 entries.

Combined molecular weight, pI and species searches select 222557 entries.

Number of sequences passing through parent mass filter: 4727

MS-Tag search selects 6 entries.

Parent mass: 1287.7700 (+/- 0.2000 Da)

Fragment ions used in search: 175.00, 255.28, 272.30, 301.48, 369.37, 432.69, 542.65, 633.34, 655.97, 742.67, 840.69 (-/- 0.70 Da)

Ion Types Considered: a b B y n h

| Search Mode | Max. # Unmatched Ions | Peptide Masses are monoisotopic | Digest Used | Max. # Missed Cleavages | Cysteines Modified by acrylamide | Peptide N terminus Hydrogen (H) | Peptide C terminus Free Acid (O H) |
|-------------|-----------------------|---------------------------------|-------------|-------------------------|----------------------------------|---------------------------------|------------------------------------|
| identity    | 2                     |                                 | Trypsin     | 1                       |                                  |                                 |                                    |

## Result Summary

| Rank | # Unmatched Ions | Sequence          | MH <sup>+</sup> Calculated (Da) | MH <sup>+</sup> Error (Da) | Protein MW (Da)/pI | Species      | NCBIInr.51403 Accession # | Protein Name  |
|------|------------------|-------------------|---------------------------------|----------------------------|--------------------|--------------|---------------------------|---|
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70288.8 / 5.29     | HOMO SAPIENS | <a href="#">8217500</a>   | bA139H14.1 (lymphocyte cytosolic protein 1 (L-plastin)) |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70289.7 / 5.20     | HOMO SAPIENS | <a href="#">4504965</a>   | lymphocyte cytosolic protein 1 (L-plastin)              |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70201.8 / 5.28     | MUS MUSCULUS | <a href="#">6679385</a>   | 65-kDa macrophage protein                               |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 32331.4 / 8.60     | MUS MUSCULUS | <a href="#">12843863</a>  | unnamed protein product                                 |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70163.8 / 5.24     | MUS MUSCULUS | <a href="#">26326929</a>  | unnamed protein product                                 |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70149.7 / 5.20     | MUS MUSCULUS | <a href="#">29840803</a>  | unnamed protein product                                 |

## Detailed Results


| Rank | # Unmatched Ions | Sequence          | MH <sup>+</sup> Calculated (Da) | MH <sup>+</sup> Error (Da) | Protein MW (Da)/pI | Species      | NCBIInr.51403 Accession # | MS-Digest Index #       | Protein Name  |
|------|------------------|-------------------|---------------------------------|----------------------------|--------------------|--------------|---------------------------|-------------------------|---|
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70288.8 / 5.29     | HOMO SAPIENS | <a href="#">8217500</a>   | <a href="#">696262</a>  | bA139H14.1 (lymphocyte cytosolic protein 1 (L-plastin)) |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70289.7 / 5.20     | HOMO SAPIENS | <a href="#">4504965</a>   | <a href="#">725402</a>  | lymphocyte cytosolic protein 1 (L-plastin)              |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70201.8 / 5.28     | MUS MUSCULUS | <a href="#">6679385</a>   | <a href="#">746520</a>  | 65-kDa macrophage protein                               |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 32331.4 / 8.60     | MUS MUSCULUS | <a href="#">12843863</a>  | <a href="#">1146923</a> | unnamed protein product                                 |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70163.8 / 5.24     | MUS MUSCULUS | <a href="#">26326929</a>  | <a href="#">1174311</a> | unnamed protein product                                 |
| 1    | 1/11             | (R)NWMNSLGVNPR(V) | 1287.6268                       | 0.1432                     | 70149.7 / 5.20     | MUS MUSCULUS | <a href="#">29840803</a>  | <a href="#">372720</a>  | unnamed protein product                                 |

| Fragment-ion (m/z) | 175.00         | 255.28                          | 272.30         | 301.48         | 369.37                          | 432.69         | 542.65         | 633.34         | 655.97         | 742.67         | 840.69 |
|--------------------|----------------|---------------------------------|----------------|----------------|---------------------------------|----------------|----------------|----------------|----------------|----------------|--------|
| Ion-type           | y <sub>1</sub> | y <sub>2</sub> -NH <sub>3</sub> | y <sub>2</sub> | b <sub>2</sub> | y <sub>3</sub> -NH <sub>3</sub> | b <sub>3</sub> | y <sub>3</sub> | b <sub>3</sub> | y <sub>6</sub> | y <sub>7</sub> |        |
| Delta Da           | -0.12          | 0.13                            | 0.13           | 0.35           | 0.18                            | 0.52           | 0.34           | 0.09           | 0.58           | 0.25           |        |

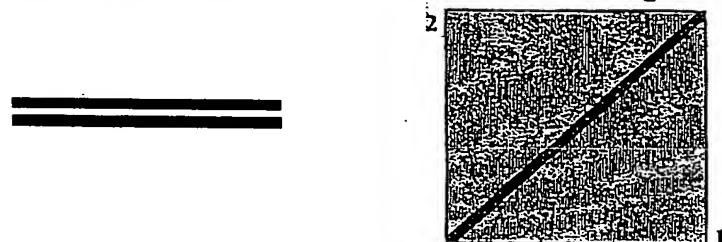
22/25

FIG. 15A

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.4 [Aug-26-2002]

Matrix: BLOSUM62 gap open: 11 gap extension: 1  
x\_dropoff: 50 expect: 10.00 wordsize: 3 Filter ☒ 

Sequence 1 gi 72220 heat shock protein 86 - mouse Length 733 (1 .. 733)  
Sequence 2 gi 72223 heat shock protein 84 - mouse Length 724 (1 .. 724)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 1102 bits (2851), Expect = 0.0  
Identities = 564/733 (76%), Positives = 611/733 (82%), Gaps = 9/733 (1%)

```
Query: 1  MPEETQTQDQPMEEEEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELISNSSDALDKIR 60
          MPEE                      EEEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELISN+SDALDKIR
Sbjct: 1  MPPEVHNG-----EEEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELISNASDALDKIR 55

Query: 61  YESLTDPSKLD SGKELHINLIPSKQDRITLIVDTGIGMTKADLINNLGTIAKSGTKAFME 120
          YESLTDPSKLD SGKEL I++IP+ Q+RTLT+VDTGIGMTKADLINNLGTIAKSGTKAFME
Sbjct: 56  YESLTDPSKLD SGKELKIDIIPNPQERTLTIVDTGIGMTKADLINNLGTIAKSGTKAFME 115

Query: 121  ALQAGADISMIGQFGVGFYSAYLVAEKVTVITKHNDDEQYAWESSAGGSFTVRDGTGPEM 180
          ALQAGADISMIGQFGVGFYSAYLVAEKV VITKHNDDEQYAWESSAGGSFTVR D GEP+
Sbjct: 116  ALQAGADISMIGQFGVGFYSAYLVAEKVVVITKHNDDEQYAWESSAGGSFTVRADHGEPI 175

Query: 181  GRGTVILHLKEDQTEYLEERRIKEIVKHSQFIGYPITLFVEKERXXXXXXXXXXXXXXXXX 240
          GRGTVILHLKEDQTEYLEERR+KE+VKHSQFIGYPITL++EKER
Sbjct: 176  GRGTVILHLKEDQTEYLEERRVKEVVKHSQFIGYPITLYLEKEREKEISDDEAEEEKG 235

Query: 241  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 300
          LNKTKPIWTR
Sbjct: 236  EKEEDKEDEEKPKIEDVGSDEEDDSGKKKKKTKKIKEYIDQEE---LNKTKPIWTR 291

Query: 301  NPDDITNBEYGEFYKSLTNDWEEHLAVKHFSVEGQLEFRALLFVPRRAPFDLFENRKKQN 360
          NPDDIT EEYGEFYKSLTNDWE+HLAVKHFSVEGQLEFRALLF+PRRAPFDLPEN+KKKN
Sbjct: 292  NPDDITQEEYGEFYKSLTNDWEDHLAVKHFSVEGQLEFRALLFIPRRAPFDLFENKKKKQN 351

Query: 361  NIKLYVRRVIFMDNCEELIPEYLNFIIRGVVDSDELPLNISREMLQQSKILKVIKKNLVKK 420
```

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FIG. 15B

NIKLYVRRVFIMD+C+ELI LNFIKGVVDSSEDLPLNISREMLQQSKILKVI VVKK  
 Sbjct: 352 NIKLYVRRVFIMDSCDELIHEYLNFIKGVVDSSEDLPLNISREMLQQSKILKVIKVNIVKK 411  
 Query: 421 CLELFTELAEDKENYKKFYEQFSKNIKLGIHEDSQNRKCLSELLRYTTSASGDEMVS LKD 480  
 CLELF+ELAEDKENYKKFYE FSKN+KLG IHEDS NR++LSELLRY+TS SGDEM SL +  
 Sbjct: 412 CLELFSELAEDKENYKKFYEA FSKVLKLG IHEDSTNRRRLSELLRYHTSQSGDEMTSLSE 471  
 Query: 481 YCTRMKENQKHIFITGETKDQVANS AFVERLRKHCL EVIYMI EPIDEYCVQQLKEFEGK 540  
 Y +RMKE OK IY+ITGE+K+QVANS AFVER+RK G EV+YM EPIDEYCVQQLKEF+GK  
 Sbjct: 472 YVSRMKETQKSIYYITGESKEQVANS AFVERVRKRGFEVVMTEPID EYCVQQLKEFDGK 531  
 Query: 541 TLVSVTXXXXXXXXXXXXXXXXXXXXXXXXXNLCKIMKDILEKKVEKVVVSNRLVTSPCCI 600  
 +LVSVT NLCK+MK+IL+KKVEKV +SNRLV+SPCCI  
 Sbjct: 532 SLVSVTKEGLELPEDEEEKKMEESKAKFENLCKLMKEILDKKVEKVTISNRLVSSPCCI 591  
 Query: 601 VTSTYGWTANMERIMKAQALRDNSTMGYMAAKIGHLEINPDHSIIETLRQKAEADKNDKSV 660  
 VTSTYGWTANMERIMKAQALRDNSTMGYM AKKHL EINPDH I+ETLRQKAEADKNDK+V  
 Sbjct: 592 VTSTYGWTANMERIMKAQALRDNSTMGYMAAKIGHLEINPDHPIVETLRQKAEADKNDKAV 651  
 Query: 661 KDLVILLYETALLSSGFSLED PQT HANRIYRMIKLG LGIDEDDPTVDDTSAAVTEEMPPL 720  
 KDLV+LL+ETALLSSGFSLED PQT H+NRIYRMIKLG LGIDED+ T ++ SAAV +E+PPL  
 Sbjct: 652 KDLVVLLFETALLSSGFSLED PQT HS NRIYRMIKLG LGIDEDVTAEP SAAVPDEIPPL 711  
 Query: 721 EGDDDTSRMEEVD 733  
 EGD+D SRMEEVD  
 Sbjct: 712 EGDEDA SRMEEVD 724

CPU time: 0.17 user secs. 0.01 sys. secs 0.18 total secs.

Lambda K H  
 0.316 0.134 0.372

Gapped  
 Lambda K H  
 0.267 0.0410 0.140

Matrix: BLOSUM62

Gap Penalties: Existence: 11, Extension: 1

Number of Hits to DB: 5349

Number of Sequences: 0

Number of extensions: 384

Number of successful extensions: 3

Number of sequences better than 10.0: 1

Number of HSP's better than 10.0 without gapping: 1

Number of HSP's successfully gapped in prelim test: 0

Number of HSP's that attempted gapping in prelim test: 0

Number of HSP's gapped (non-prelim): 1

length of query: 733

length of database: 405,742,523

effective HSP length: 134

effective length of query: 599

effective length of database: 405,742,389

effective search space: 243039691011

effective search space used: 243039691011

T: 9

A: 40

X1: 16 ( 7.3 bits)

X2: 129 (49.7 bits)

X3: 129 (49.7 bits)

S1: 41 (21.6 bits)

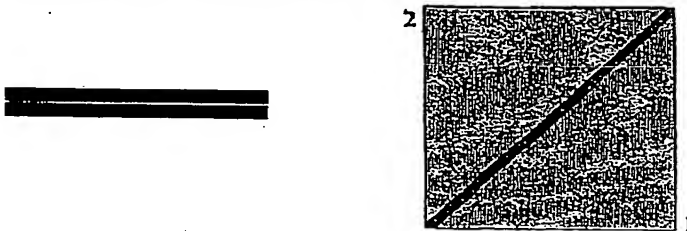
S2: 78 (34.7 bits)

FIG. 16A

Matrix **BLOSUM62** gap open: **11** gap extension: **1**  
 x\_dropoff: **50** expect: **10.00** wordsize: **3** Filter **F**

Sequence 1 gi 72220 heat shock protein 86 - mouse Length 733 (1 .. 733)

Sequence 2 gi 72223 heat shock protein 84 - mouse Length 724 (1 .. 724)



NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score = 1102 bits (2851), Expect = 0.0

Identities = 564/733 (76%), Positives = 611/733 (82%), Gaps = 9/733 (1%)

```

Query: 1  MPEETQTQDQPMEEEEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELISNSSDALDKIR 60
          MPEE                      EEEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELIS+SDALDKIR
Sbjct: 1  MPPEVHHG-----EEEVETFAFQAEIAQLMSLIINTFYSNKEIFLRELISNASDALDKIR 55

Query: 61  YESLTDPSKLDGKELHINLIPSKODRTLIVDTGIGMTKADLINNLGTIAKSGTKAFME 120
          YESLTDPSKLDGKEL I+IP+ Q+RTLT+VDTGIGMTKADLINNLGTIAKSGTKAFME
Sbjct: 56  YESLTDPSKLDGKELKIDIIPNPQERTLTIVDTGIGMTKADLINNLGTIAKSGTKAFME 115

Query: 121  ALQAGADISMIGQFGVGFYSAYLVAEKVTVITKHNDDEQYAWESSAGGSFTVRDGEPM 180
          ALQAGADISMIGQFGVGFYSAYLVAEKV VITKHNDDEQYAWESSAGGSFTVR D GEP+
Sbjct: 116  ALQAGADISMIGQFGVGFYSAYLVAEKVVVITKHNDDEQYAWESSAGGSFTVRADHGEPI 175

Query: 181  GRGTKVILHLKEDQTEYLEERRIKEIVKHSQFIGYPITLFVEKERXXXXXXXXXXXXXXXXX 240
          GRGTKVILHLKEDQTEYLEERR+KE+VKHSQFIGYPITL++EKE
Sbjct: 176  GRGTKVILHLKEDQTEYLEERRVKEVVKHSQFIGYPITLYLEKEREREISDDEAEKEG 235

Query: 241  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 300
          LNKTKPIWTR
Sbjct: 236  EKEEDKEDEEKPKIEDVGSDEEDDSGDKKKKTKKIKKIKYIQEE----LNKTKPIWTR 291

Query: 301  NPDDITNEEYGEFYKSLTNDWEHLAVGHFSVEGQLEFRALLFVPRRAPFDLFENRKKKN 360
          NPDDIT EEEYGEFYKSLTNDWE+HLAVKHFSVEGQLEFRALLF+PRRAPFDLFEN+KKKN
Sbjct: 292  NPDDITQEEYGEFYKSLTNDWEDHLAVKHFSVEGQLEFRALLFIPRRAPFDLFENKKKN 351

Query: 361  NIKLYVRRVFIMDNCEELIPEYLNFIIRGVVDSDELPLNISREMLQQSKILKVIRKQNLVKK 420

```



25/25

FIG. 16B

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```

NINLYVRRVFIND+C+EL. LNFIRGVVDSDELPLNISREMLQOSKILKVI +VKK
Sbjct: 352 NINLYVRRVFINDSCDELIREYLNFRGVVDSDELPLNISREMLQOSKILKVI+ANIVKK 411
Query: 421 CLELFTELARDKENYKKFYEPFSKXIKLGIHEDSQNRQCLSELLRYTTSAGDEMVSLEK 480
      CLELF+ELAEDKENYKKFYEPFSKX+KLGIEHDS NR++LSELLRY+TS SGDEM SL +
Sbjct: 412 CLELPSELAEDKENYKKFYEPFSKXIKLGIHEDSTNRRRLSELLRYHTSOSDENTSLSL 471
Query: 481 YCTAMKENGKHIYFITGETKQVANSATVERLRKHCLEVIYMIPIDEYCVQQLKEFEGR 540
      Y+RMKE OK IY+ITGE-X+QVANSATVER-RK G-EV-YM EPIDEYCVQQLKEF+CK
Sbjct: 472 YVSRHKAOKSIXYITGESKQVANSATVERLRKHCLEVIYMIPIDEYCVQQLKEFEGR 531
Query: 541 TLVSVTXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 600
      +LVSVT NICK+MK+IL+KKVERV +SNRLV+SPCCI
Sbjct: 532 SLVSVTKEGLELPEDEEEKKQMEESKAKFENLCKL+KKEITLDKKVEKVTISNRLVSSPCCI 591
Query: 601 VTSTYGTAMMERIMKAQALRDNSTMGYMAKQKLEINFDHSIITLROKAEADKNDKSV 660
      VTSTYGTAMMERIMKAQALRDNSTMGYMAKQKLEINFDH I+ETLROKAEADKNDK+V
Sbjct: 592 VTSTYGTAMMERIMKAQALRDNSTMGYMAKQKLEINFDHPIVETLROKAEADKNDKAV 651
Query: 661 KDLVILLIETALLSSGFSLEDPQTHANRIYRMINKLGLGIDEEDPTVDTSAAVTEEMPLL 720
      KDLV+LL+ETALLSSGFSLEDPQTH+NAIYRMINKLGLGIDEED+ T++SAAV +E+PPL
Sbjct: 652 KDLVILLIETALLSSGFSLEDPQTHSNRIYRMINKLGLGIDEEDVTAEPSSAAVDPDEIPLL 711
Query: 721 EGDDETSRMEEVD 733
      EGD+O SRMEEVD
Sbjct: 712 EGDDETSRMEEVD 724

```

CPU time: 0.17 user secs. 0.01 sys. secs 0.18 total secs.

```

Lambda      K      H
0.316      0.134    0.372
Gapped
Lambda      K      H
0.267      0.0410   0.140

```

```

Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 5349
Number of Sequences: 0
Number of extensions: 184
Number of successful extensions: 3
Number of sequences better than 10.0: 1
Number of HSP's better than 10.0 without gapping: 1
Number of HSP's successfully gapped in prelim test: 0
Number of HSP's that attempted gapping in prelim test: 0
Number of HSP's gapped (non-prelim): 1
length of query: 733
length of database: 405,742,523
effective HSP length: 134
effective length of query: 599
effective length of database: 405,742,389
effective search space: 243039691011
effective search space used: 243039691011
T: 9
A: 40
X1: 16 (7.3 bits)
X2: 129 (49.7 bits)
X3: 129 (49.7 bits)

S1: 41 (21.6 bits)
S2: 78 (34.7 bits)

```